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Changing Concepts of Prolactin in Man

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THAT THERE IS A HUMAN PROLACTIN, an entity separate and distinct from growth hormone, was until recently the firm conviction of the few. Progress has been so rapid that the year 1972 will almost surely be the last to see an authoritative publication appear in which doubt of the existence of human prolactin is either implied or expressed.[1] This article will summarize the important and rapidly accumulated evidence which justifies the statement that its existence is now a secure fact.

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In writing on "Changing concepts of control of human growth hormone secretion"[2] I avoided the problem of prolactin. Human growth hormone for more than a decade has been recognized to have unusually great prolactin activity when tested in the pigeon crop sac bioassay or especially in mammary gland bioassays.[3] To have discussed the control of growth hormone as a single molecule having a dual function, the second of which was the sole pituitary prolactin, I should have found impossibly difficult.

Controversy about growth hormone and prolactin is not at an end, and one of the points firmly believed by our group of investigators is that human prolactin behaves just as it should; human growth hormone, in contrast, is peculiar among

the growth hormones of mammals. It is this fact which slowed the recognition of human prolactin. A brief exegesis may be helpful.

A lot has been made of the structural resemblance of sheep prolactin to human growth hormone; electrophoretic similarities and even structural homologies have been stressed. This approach has not suffered from understatement. The facts are probably more accurately portrayed by stating that human prolactin is much like sheep prolactin;[4] indeed there is about 75 percent homology in the amino acid sequence of the two molecules according to this study. Most prolactins are more acidic than their corresponding growth hormones. Human growth hormone is peculiarly acidic, so much so that on disc electrophoresis it migrates ahead of human prolactin.[5] Human growth hormone is also peculiarly active as a lactogen. An epexegesis, for which I am indebted to Dr. U. J. Lewis, will follow, but the foregoing will orient the reader to a point of view.

Morphological Identification of Human Prolactin

The first essential for the identification of a new hormone is a willingness to concede its existence. Morphologists, to their great credit, were the first to exceed this minimal requirement; they pressed for the recognition of human prolactin, because they recognized a cell line with special staining characteristics. Three staining methods have been developed by which prolactin-secreting cells could be postulated. These are Herlant's tetrachrome, his methasol blue-PAS-orange G, and in 1968 Brook's stain.[6-8] Histological techniques suggested the presence of prolactin secretory granules in the tissue cultures of pituitary cells,[9] and in pregnancy pituitary cells were rich in these special staining cells.[10,11] Finally, special stains pointed to prolactin-secreting cells in certain pituitary tumors.[12] It is notable that the percentage of prolactin secreting cells in pregnancy reaches as high as 18 to 32.

Pasteels and colleagues,[13,14] convinced by the histological evidence, pursued prolactin production by human pituitary glands in culture. They amassed substantial data favoring production of a pituitary prolactin by cultures made from glands either of adult or of fetal origin. The cells with the staining characteristics implying prolactin production multiplied in the cultures; the proteins in the medium were rich in crop sac stimulating activity, and growth hormone secreting cells tended to drop out. Pasteels[15] showed that antiserum raised against human growth hormone did not neutralize the pigeon crop sac stimulating activity of the proteins secreted into the medium. Unfortunately the prolactin activity assayed only 2.95 IU per mg, about 10 percent the activity of a good sheep prolactin preparation. This was used as an antigen in one of the early radioimmunoassays attempted,[16] and some useful observations were possible with it. In my opinion, Pasteels more than any other investigator deserves to be credited with pioneering the identification of human prolactin.

Before leaving the histologic aspects, some remarks on pituitary cell nomenclature may prove useful, particularly for clinical consideration. Classifying pituitary tumors as acidophilic, basophilic, or chromophobic no longer fulfills the need for maximal accuracy. Nomenclature should relate more nearly to function. The established function of the anterior pituitary is to synthesize, store, and release six hormones. Separate cell lines secrete these hormones, and the goal should be a physiologically-based classification. Three hormones are glycoproteins, and these are thyrotropin (TSH), follicle stimulating hormone (FSH) and luteinizing hormone (LH or ICSH). The remaining three hormones contain no sugars, and their primary structure is that of a sequence of amino acids, 190 for human growth hormone and probably for prolactin, and 36 for corticotropin (ACTH). LH and TSH are each composed of two chains. Structural analysis has demonstrated that their B chains are identical, and this requires special precautions in radioimmunoassays. In the prolactins thus far studied there are six half-cystines, and these give rise to three disulfide bridges. Growth hormones have two disulfide bridges. Staining characteristics in modern work should be based on the affinities of the dyes for the specific hormones. These hormones under ordinary circumstances are present as secretory granules. However, when secretion is at a high rate little hormone is stored, and that cell line will be "degranulated." There appear to be resting cells in the pituitary gland, and they, too, will be poor in secretory granules. "Acidophils" are a combination of growth hormone, prolactin and ACTH secreting cells. "Basophils" generally contain glycoprotein hormonal granules, but intense cellular activity will be associated with sufficiently great numbers of ribosomes to give a basophilic reaction to the cytoplasm. "Chromo-

phobes" are a mixture of very active cells with few storage granules and of resting cells. Rarely are chromophobes truly undifferentiated cells. These considerations are pertinent, because it turns out that prolactin is probably the most commonly overproduced hormone in pituitary tumors.

Physiochemical Identification of Human Prolactin

The introduction of sensitive radioimmunoassays for human growth hormone made it possible to divorce galactorrhea in certain circumstances from growth hormone excess and even from measurable growth hormone in plasma. This implied the possibility of prolactin excess as cause of the breast secretion. Canfield and Bates[17] demonstrated stimulation of the pigeon crop sac by extracts made from the plasmas of five non-acromegalic patients with galactorrhea. Only one such extract was inactive. These observations, particularly when the extracts were made from blood of patients with pituitary tumors, implied that there was a separate prolactin. More direct evidence was supplied by Peake et al[18] when they found evidence of prolactin in a tumor removed from a patient with Forbes-Albright syndrome (galactorrhea and amenorrhea with or without pituitary tumor). The positive crop sac response, in contrast to that to human growth hormone, was not prevented by previous incubation with antiserum raised against human growth hormone. Further, using the disc electrophoretic method developed for studying growth hormone and prolactin in rats by Jones et al,[19] they demonstrated a major band moving more slowly than growth hormone. They clearly recognized they had not shown this to be the prolactin band and commented that the relative mobilities were unusual. These authors also cited, in support of the idea of a pituitary prolactin separate from growth hormone, the studies of Ehni and Eckles and those of Field et al.[20,21] These were neurosurgical reports that pituitary stalk section resulted in galactorrhea despite diminution of growth hormone secretion.

The foregoing reports were highly suggestive of a separate human prolactin, but they were all made in abnormal situations and open to another interpretation by those devoted to the unified growth hormone-prolactin theory. The counter argument could run to the effect that in disease only the prolactin moiety of the molecule was made.

In our laboratories the strong interest of Lewis had not only led to involvement in evolutionary aspects of structure and function of growth hormones and prolactins but also to conviction that the new clues in man pointed to a separate prolactin. Our earlier studies with Jones[19] centered our interest on obtaining pituitary glands from patients who had been receiving (or making) estrogens at the time of death; for we had found with disc electrophoresis that the "prolactin band" of the pituitary was much larger in mature female rats than in males and that this was an estrogen effect. Through the kind helpfulness of Dr. Averill Liebow and his staff at University Hospital, San Diego, we were provided with the opportunity to study postmortem the pituitary of a woman who had been taking estrogen and that of one who had died just after the termination of pregnancy. From these glands it was possible to isolate the protein of a molecular weight of 20,000 Daltons, a prolactin activity of at least 15 I.U. per mg, and to demonstrate a new constituent of the normal pituitary, a protein band moving on disc electrophoresis just behind human growth hormone.[5] More important, this study provided a marker in disc electrophoresis for following prolactin in the extraction of large batches of frozen human pituitary glands. These were kindly supplied by the National Pituitary Agency and enabled Dr. U. J. Lewis and his group to extract human prolactin in highly purified form for further identification and for structural studies.[4,22]

At the same time, Friesen and his colleagues in Montreal were making an excellent series of important advances in the areas of monkey and human prolactins. Their studies are summarized in a chapter of Human Lactogens,[23] but unfortunately this volume is not widely available. A brief account of these crucial studies will be attempted. Friesen and his group cultured pituitary glands of monkeys, normal human pituitary glands obtained at operation for breast cancer, glands from acromegalic patients and from patients with galactorrhea. These pituitary fragments in culture secreted proteins into the media. Radioactive leucine, introduced into the culture fluid, was incorporated into the proteins and served to label them. The remarkable finding was that gel filtration of the media not only resulted in a single large radioactive peak but also that an antibody raised against human placental lactogen or growth hormone precipitated much less of this material than did antibody to sheep prolactin. This supported their inference that

the human pituitary gland, like that of animals, secreted prolactin and that there was some structural similarity of this human hormone to sheep prolactin.[24,25] Tumors from patients with galactorrhea were found to secrete into the medium preponderantly prolactin, whereas glands from acromegalic patients tended to secrete much more growth hormone. For purification studies the technique of affinity chromatography was then pursued. In this procedure antibody is coupled to Sepharose; when the labeled protein mixture was passed over the column, labeled prolactin or labeled growth hormone was bound according to which antibody had been coupled to the Sepharose. They were clearly able to prepare human prolactin in small quantities but of good activity (13 I.U. per mg) and with very little contamination. In 1972, they presented their method for preparing human prolactin of high activity from a side fraction of the Raben procedure for making human growth hormone.[26] This preparation and that of Lewis gave virtually identical inhibition when tested by their radioimmunoassay.[27]

Biochemistry of Human Prolactin

The first published methods for the extraction of human prolactin were those of Lewis et al[4,22] and of Hwang et al.[26] In the Lewis procedure the starting material was frozen human pituitary glands. The final product was about 5 micrograms of prolactin per gland. Its potency in the pigeon crop sac assay was about 30 I.U. per mg, comparable to sheep prolactin potency of the National Institutes of Health (NIH) standard. Success required a means of following the prolactin component through the extraction procedure, and this was made possible by the findings on disc electrophoresis already noted. A second requirement was mastering problems of hormonal instability during extraction. This is a trying problem because of the low concentrations of prolactin in the starting material. Glands stored frozen clearly lose their prolactin. A perhaps more intriguing problem is that human growth hormone undergoes specific cleavage during storage and extraction, and this cleavage is attended by enhanced prolactin activity in crop sac assays. The cleavage does not, however, lessen growth hormone activity by tibial test or by radioimmunoassay. This enhancement of prolactin effect by this means is peculiar to human growth hormone.

The method now in use[4] goes back to the original ethanolic extraction procedure by which Bates and Riddle first isolated sheep prolactin in 1935. Freeing the partially purified prolactin of contamination with growth hormone can be accomplished chromatographically by a variety of techniques, including diethylaminoethyl (DEAE) cellulose and carboxy methylcellulose. The preparative method of Hwang et al, using the Raben extraction by-product, appears to yield a highly suitable human prolactin.[26]

The primary structure of human prolactin, that is, in terms of its amino acid sequence, has already been approached by two methods. Lewis et al,[4] using the method of tryptic digestion and amino acid analysis of the peptides isolated on two-dimensional paper chromato-electrophoretic separation (finger-printing), have proposed about 80 percent of the human prolactin structure. They found a molecular weight of 20,000 Daltons, a leucine amino terminus, three disulfide bridges, and about 75 percent homology with ovine prolactin. This type of sequence prediction depended on the known structure for ovine prolactin.[28] At the same time Niall,[29] in collaboration with Friesen, analyzed the amino terminal portion of the molecule, using the methods of Edman degradation, cyanogen bromide and tryptic cleavage. This exacting procedure, which consumes much more prolactin, is in such good agreement with the finger-printing method that it appears highly probable the estimate of 70 to 80 percent homology between human and ovine prolactins will prove to be fully justified.

The evidence to date speaks for the similarities in the structures of prolactins of human, ovine and bovine origins. If closer similarities between mammalian prolactins than growth hormones turn out to be the general rule when more structure data are at hand, there will already be an exception. Thus, rat and mouse prolactins almost surely have far less structural resemblance than the corresponding growth hormones. The most remarkable aspect of structure-to-function relationship is that which exists between human placental lactogen and human growth hormone.[30] The former is primarily lactogenic and a weak growth hormone. It does little or nothing to potentiate the growth effects of growth hormone. However, there is a pronounced similarity of the structures of these two hormones, evidence which probably should be taken to testify to the uniqueness of human growth hormone.

Measurement of Human Prolactin

Homologous radioimmunoassays for human prolactin are now available.[31,32] We have supplied materials for this assay to the National Pituitary Agency for distribution for research purposes. There can be little doubt that this method will be one of choice for the measurement of prolactin in blood and other body fluids. Nonetheless, very important advances in the understanding of prolactin secretion in health and disease were made with ingenious bioassay procedures of surprising sensitivity; none measured prolactin in the concentrations found in normal human blood. Kleinberg and Frantz[33] devised an assay based on histological evidence of milk production by the cultures of mammary tissue taken from mice two weeks pregnant. Prolactin in plasma of women could be detected by this assay, and elevations induced by lactation, by drugs, and in abnormal states were clearly defined. Human growth hormone also stimulated the mammary tissue, but this difficulty was obviated by neutralizing the growth hormone in plasma by addition of specific antibody. Loewenstein et al[34] devised an assay based on activation of a synthetase for n-acetyllactoseamine, and Turkington[35] found mammary tissue sufficiently sensitive to prolactin that incorporation of[32] phosphorus into milk proteins provided a suitable bioassay system. In England, Forsyth used a rabbit mammary gland culture system, capable of measuring prolactin in blood of mothers after suckling and of some patients with non-puerperal lactation. A scholarly article by Forsyth and Edwards[36] has just appeared, reviewing the field and the numerous contributions of these workers.

Heterologous radioimmunoassays are capable of measuring prolactin in human blood.[37] Here the structural similarity of ovine to human prolactin is often the essential factor. As in homologous assays, antibody is raised in rabbits, but in this case ovine prolactin is used as the antigen. The antibody so raised has been most successfully employed to bind radioiodinated porcine prolactin.[38] Inhibition of this binding was found with a human plasma rich in prolactin, enabling a standard curve to be constructed. In another heterologous assay Friesen et al[27] induced antibody in rabbits against monkey prolactin and iodinated sheep prolactin. When they substituted primate prolactin for iodination, the result was a great increase in the sensitivity of their assay.

Because it gives an intimation of structural differences in terms of immunoreactive sites, special interest attaches to the cross-reaction of the prolactins of lower species in the homologous human radioimmunoassay for prolactin. The question here is what prolactins of lower species have sufficient similarity to human prolactin to inhibit the binding of the labeled human prolactin to an antibody raised against it in rabbits. In our totally homologous system, ovine and bovine prolactins inhibited binding of human prolactin to its antibody to a considerable extent. In the jargon of the field, the curves of inhibition were not parallel, suggesting that these prolactins were sufficiently different structurally in the critical areas that affinities between them and the antibody differed from that for the human hormone. Mouse prolactin in the radioimmunoassay gave a weaker nonparallel inhibition, and rat, pig, whale, turtle, frog and shark prolactins did not cross-react at all. Interestingly and in agreement with Friesen's Montreal group, we found no inhibition by human placental lactogen. Also in agreement with them, we found human growth hormone to cross-react only at very high concentration and interpreted this to be a measure of the degree to which human growth hormone was contaminated by human prolactin.

In practical terms, these studies mean that human prolactin can be measured accurately in blood despite maximal levels of growth hormone, such as are found in acromegaly. Further, human placental lactogen offers no obstacle to the measurement of human prolactin at any point in pregnancy. This is in contrast to the situation which is found with growth hormone which cannot be measured with any ease in pregnancy because of the cross reactions between it and human placental lactogen. Again this reflects the peculiarity of human growth hormone, its structural similarity to a lactogen and its own innate lactogenic activity.

One other point deserves elaboration. Despite the failure of pig prolactin to inhibit binding of human prolactin by the latter's specific antibody, pig prolactin can be used for radioiodination in the heterologous assay, as mentioned. Binding of this pig prolactin to antibody raised against sheep prolactin is inhibited by human prolactin. Since both pig and sheep prolactins have been available for years, the necessary ingredients for a sensitive heterologous assay have been on hand for a considerable time, substantially longer than there has been agreement on the existence of human prolactin as an entity.

Physiology of Human Prolactin

Blood drawn from ambulatory adults, men and women, contains on the average about 10 nanograms prolactin per ml. The range is not defined. The influences of age, sex, time of day, relation to meals and to sleep have not yet been fully explored. Friesen[39] reports the newborn to have pronounced elevations of prolactin at birth and continuing for a week (200 ng per ml falling to 50). The functional significance of this is as obscure as it is intriguing. Men, in whom the function of prolactin is almost entirely to be worked out, have about the same blood concentrations of prolactin as women. The luteal phase of the menstrual cycle may be marked by a slight rise in mean prolactin levels; if so, it appears not to exceed a 10 percent rise.

Sassin et al[40] and L'Hermite et al[41] have reported nychthemeral changes in secretion of prolactin. There is a sleep release of prolactin, but much remains to be investigated concerning its nature. Parker and Rossman[42] have found that daytime naps are associated with rises in prolactin concentrations in blood. They found with frequent bleeding that there is a broad swing in these values in around-the-clock studies, indicating the need for caution in assigning normal values and even in evaluating stressful and pharmacological influences.

Pregnancy is associated normally with a progressive rise in plasma concentration of prolactin. A fair rule of thumb for the present is that prolactin concentrations double in the first trimester and again in each subsequent trimester, finally rising at term to about 200 ng per ml.[39]

The nursing mother when not being suckled has a 10- or 20-fold elevation of concentration of prolactin in blood during the early part of the puerperium. There is a sharp rise with the suckling stimulus so that the concentration of prolactin is further doubled for some 30 minutes. As time progresses, the basal elevations and the responses to suckling are less in magnitude but each appears to be measurable over several months.

A somewhat surprising stimulus to the secretion of prolactin is the thyrotropin releasing factor or hormone (TRH). The rise is prompt, often maximal in 15 to 30 minutes after intravenous doses of TRH as low as 10 mcg. In clinical testing 200 to 500 mcg of TRH is commonly employed. Rises to 50 ng per ml and substantially higher are common. Interestingly, as with thyrotropin, prolactin is released by TRH to greater heights and for a longer time in hypothyroidism.[43,44] TRH has reduced effectiveness in releasing both prolactin and thyrotropin in Graves' disease. Physiological significance for the release of prolactin by TRH cannot be claimed. It is notable, however, that galactorrhea occurs in an occasional patient with hypothyroidism.

In lower animals prolactin secretion is generally held to be unique among the pituitary hormones. Prolactin, it is held, is released by the pituitary gland unless the release is prevented by the hypothalamus through its "prolactin-inhibiting-factor." There is support for this concept, including evidence from man. Ectopic location of the anterior pituitary or section of the pituitary stalk appears to release the prolactin secreting cells from the tonic inhibition of the hypothalamus. Stalk section certainly appears to have this effect in man.[45]

The full account of control of human prolactin secretion remains to be worked out, but it seems clear that the catecholamines are important in the pathway of release. One use of the TRH studies of prolactin release is to enable a reasonable calculation of the disappearance rate of prolactin from plasma. In studies we have done in collaboration with Dr. Inder Chopra of the University of California, Los Angeles, Department of Medicine, it repeatedly appeared that in 15 minutes prolactin concentration declines its peak to a concentration 50 percent or more below the peak. We have seen a half-time of less than 10 minutes in this type of study with TRH. This is in keeping with figures stated by others for the disappearance rate of this hormone from blood. The rapidity of disappearance suggests that the effect of TRH on the prolactin secreting cells is of very brief duration.

A physiological role for prolactin in lactation is beyond doubt. The nature of the receptor proteins of mammary tissue and the translation of the hormonal stimulus to enzymic action is already unfolding; Turkington and his colleagues have recently summarized their many contributions on this topic.[46] That lactation has been essential to the long term survival of man would seem beyond reasonable doubt and a sufficient reason for there being a prolactin. The role of the prolactin in such high concentration in the newborn and the presence of the hormone in the male pose solid unanswered problems. Fascinating suggestions in consideration of these roles are found in lower forms. The euryhaline fishes require an intact pituitary for maintenance of salt and water balance in going from sea water to fresh water,

and prolactin restores the defect that hypophysectomy induces in this balance. It appears that hens bred for egg-laying capacity have had prolactin bred out of them, and cows bred for milk production have high concentrations of prolactin. The former are broody under the influence of prolactin; the latter, especially the prize milk producers, are susceptible to a bizarre behavioral disorder which is accompanied by failure of lactation.[47] Here the role of prolactin is uncertain. In some rodents prolactin is luteotrophic and essential for the maintenance of pregnancy, a role not played by this hormone in human beings. Prolactin is "antigonadal" in some respects, and this may be a significant function for human prolactin. Nursing mothers are so protected against conception that subsequent pregnancies are delayed nearly one year without contraceptive measures. The method is not fully reliable.

In the male newt prolactin augments the effect of testosterone on the nuptial pad, a device for clasping the female during amplexus. In the male mouse prolactin plays a role in fertility. In the male rat prolactin enhances the effect of testosterone on the prostate, and induction of binding sites for testosterone has been described in the human prostate.

Another line of evidence of nonlactational effects of prolactin is found in the studies of ovine prolactin in man. The abundance of ovine prolactin, its rather superficial resemblances to human growth hormone and the scarcity of the latter, clearly prompted this type of study. Although this bias slanted the selection of the physiological effects to be observed, the newer evidence of a strong homology between the prolactins of man and sheep suggests that the effects observed after injection of sheep prolactin be regarded primarily as clues to the mode of action of human prolactin. One difficulty with the acceptance of this approach is that the ovine prolactin was injected in individuals without knowledge of their endogenous prolactin concentration, but this reservation would apply chiefly where no responses were noted. Further, the homology is incomplete, and the studies therefore provide clues rather than proofs. For these and some additional reasons, quantitative comparisons of human growth hormone effects and ovine prolactin effects probably should not be made.

If we accept the hypopituitary and the hypophysectomized patients as most suitable for these studies, the following effects have been found with reasonable frequency after ovine prolactin: fall in blood urea nitrogen, increased urinary calcium and phosphorus excretion, glucose intolerance, and occasional increase in sodium excretion. In balance studies nitrogen balance has tended to be positive.[48] Nitrogen retention has also been observed regularly in the few cases treated with bovine prolactin, and interestingly in view of what has been said about structure, pig prolactin has caused decidedly negative nitrogen balance.[49] Although in our laboratory the yields of human prolactin have been improving substantially with better storage and extraction methods, we see little hope for human prolactin's being available in the requisite amount for metabolic studies in the near future. Pharmacological and clinical clues to the mode of action of prolactin in man and the use of homologous prolactin in animals should, however, provide new evidence of the physiological role of prolactin.

Pharmacology of Human Prolactin Secretion

Fairly extensive studies in the rat, well-known to have high concentrations of hypothalamic catecholamines when compared with the brain generally, show that depletion of hypothalamic catecholamines by drugs results in elevation of blood prolactin content. This appears to occur because of a reduction in prolactin-inhibiting factor. Further, drugs which elevate the catecholamine content of the hypothalamus lower blood prolactin levels. For many years it has been observed that psychotropic drugs, such as chlorpromazine, result in galactorrhea. The frequency with which milk appears in the breast varies with the configuration of the drug, dosage and duration of administration. Apostolakis et al have recently recorded an extensive experience with these drugs, used singly and in combination, for their effects in producing galactorrhea.[50] These drugs deplete the hypothalamus of catecholamines, and the effect of chlorpromazine can be used to test for prolactin secretory capacity of the pituitary gland.[51] In this test 25 or 50 mg of chlorpromazine injected intramuscularly results, in one to two hours, in 10- to 20-fold rises in the blood concentration of prolactin. Chlorpromazine causes hypotension and should be used cautiously for this purpose. As mentioned above, TRH also releases prolactin. This effect is of unknown physiological significance, but the apparent lack of side-reaction to this tripeptide may make it valuable as a drug in testing for prolactin release. Reserpine, alpha-methyl-*p*-tyro-

sine, methyl dopa, and d-amphetamine are among the agents which deplete catecholamines. Their place is not yet determined as pharmacological agents for the study of pituitary reserve. Reserpine as a galactorrheic agent which enhances prolactin secretion is clearly recognized.

In the studies of Turkington,[52] L-tryptophan given in 10-gram dosage over 20 minutes intravenously causes a prompt, many-fold release of prolactin. Presumably this action depends on the formation of hydroxytryptophan and possibly serotonin. In contrast to tryptophan, L-dopa suppresses prolactin secretion in man, and this pharmacological effect of levodopa has been used both in testing and in treatment.[27,38,53] Dosage has ranged from 250 mg three times a day to 500 mg four times a day. Prompt cessation of lactation with prolonged effectiveness of the medication and with resumption of menses in some instances has been observed. It is probable that the effect of L-dopa is on the hypothalamus to promote the release of the prolactin-inhibiting factor. Since dopamine may be a releasor of the gonadotropins, the resumption of menses may not depend on the suppression of prolactin but rather represent a direct effect of catecholamines on the luteinizing hormone releasing factor. In my opinion, this remarkable effect of L-dopa raises the possibility that the hypothalamus, normally rich in catecholamines, may be subject to a disorder of biogenic amine metabolism, with multiple or variable manifestations. This would be rather imperfectly analogous to the thalamic disorder in paralysis agitans.

A good deal of interest exists in synthesizing derivatives of ergot as suppressors of prolactin secretion. This effect has been documented in rats, and a very preliminary report[54] indicates some success in galactorrhea with a brominated ergocryptine. Prolactin excess was not demonstrated, however, and the drug was not well-tolerated in this limited experience. Forsyth et al[36] have had favorable experiences with ergot derivatives for suppressing prolactin secretion. Action here is said to be directly on the pituitary gland.

Clinical Significance of Prolactin

Although so much remains to be explored, it is already clear that the measurement of prolactin in the body fluids of man will not only be of great physiological interest but also of great clinical import. Hypersecretion of prolactin as the most common abnormality in pituitary tumors seems almost certainly to be beyond dispute. Friesen, for example, with his colleagues[39] states that it is mandatory to measure prolactin in all pituitary tumor patients. In support of this contention, he cites evidence that whereas growth hormone hypersecretion characterizes 15 to 20 percent of pituitary tumor the figure is about 30 percent for prolactin. In our studies[32] the incidence of elevated prolactin secretion in acromegaly is higher than in Friesen's experience, so that he may have underestimated the frequency with which prolactin hypersecretion occurs in patients with pituitary tumors. The more important point is that prolactin hypersecretion in his study occurred with pituitary tumor frequently when there was no inappropriate lactation. Indeed, only one third of the patients with pituitary tumor and elevation of plasma prolactin had galactorrhea.

It must be acknowledged, however, that pituitary tumor is of fairly infrequent occurrence and that galactorrhea as inappropriate lactation is arbitrarily defined and of uncertain incidence. It appears not to be known at what point after the cessation of nursing expressible secretion should disappear. Indeed the fact, as presented by Daughaday's group at the Fourth International Congress of Endocrinology, that the male responds to nipple stimulation by his wife with distinct elevation of plasma concentration of prolactin suggests that continuing induction of specific protein kinases in the human female breast in response to prolactin may better be considered in terms of sexual behavior than too readily regarded as pathological. The larger questions involve the role of prolactin in disease. In the mouse, virus induced mammary cancer and, in the rat, chemically induced mammary cancer have been shown to be prolactin-dependent. It is already apparent that human breast cancer, however, is not characterized by elevation of plasma prolactin concentrations as determined in randomly drawn samples. This statement by no means should be taken to exclude prolactin from a role. The complexity of the problem is indicated by the number of hormones that act on the breast—insulin, cortisol, thyroid hormones, prolactin, growth hormone, placental lactogen, estrogens, and progesterone. Forsyth and Edwards in their comprehensive essay on human prolactin[36] have made an extension to this point of the studies of Meir et al,[55] who showed that whether prolactin induces crop sac responses or lipogenesis in the pigeon depends on a phasic relationship to corticosterone. Eighteen hours after corticosterone injection, prolactin induces crop

sac responses: six hours later it promotes lipogenesis in liver and gut. With so many pituitary hormones showing normal, independent rhythms, with diurnal variation in responsiveness to insulin, and with the cyclic nature of the female gonadal steroid secretion, it is apparent that the endocrinology of mammary tumorigenesis may pose most complex problems. In addition, if the mouse disorder is a model for the human disease, the virus may complicate the problem, even in terms of its endocrinology. We have developed mouse growth hormone and prolactin radioimmunoassays[56,57] to pursue the study of the mouse as a model for breast cancer. It is already clear that the pituitary and blood concentrations of these hormones differ between the C_3H/St strain which transmits the tumor virus in the milk and the $C_{57}BL$ strain which is tumor-free and does not transmit the mammary tumor virus in the milk. Further, the switching of litters to nursing dams of the opposite strain is followed by significant changes in the pituitary concentrations of these hormones. Genetic and nutritional factors, as well as the virus, may have roles in these experiments. Clinical underscoring of the complexity of the problem comes from two apparently contradictory observations. L-dopa is thought to have had some beneficial effects on metastatic breast cancer, presumably through prolactin suppression. Stalk section, releasing the pituitary from the influence of prolactin-inhibiting factor and favoring prolactin secretion, has also been found beneficial. In addition, hypophysectomy which should lessen or abolish pituitary secretions has had significant beneficial effects in metastatic breast cancer. The contradictions may be only apparent, for the rhythms characterizing secretion of the involved hormones will be altered by these maneuvers. As stated in the earlier review,[2] patients with breast cancer have paradoxical releases of growth hormone in response to glucose loading, which also suppresses corticotropin. The full consequences of such a disruption of the normal secretory pattern are at present unknown. It would seem biologically important that in man growth hormone is secreted in the early hours of sleep, prolactin and thyrotropin later in sleep and in the early waking hours. Meantime, plasma cortisol concentrations are minimal during the time when growth hormone is actively being secreted, and plasma cortisol is at about maximum when the prolactin and thyrotropin are at peak concentrations. Sometime later the thyroid will have responded to thyrotropin by secreting thyroid hormones. Quite apart from the remaining hormonal influences on the breast, it seems reasonable to expect that disrupting the sequence may play both pathogenetic and therapeutic roles.

Whereas the breast remains a focus of medical interest in the role of prolactin in health and disease, we may only ask questions about other structures on which prolactin may have important physiological or pathological bearing. As a hormone that affects behavior in animals, is it related to mood in man? Is its sodium retaining activity of major significance in certain pathological states? Why is it in high concentration in the blood of the newborn—and in amniotic fluid? Does it play an unexpected role in much of amenorrhea? Does it have a relationship to diabetes? What does it do in men? The past two years have been exciting for those interested in endocrinology; the next two years should see better questions asked. Better answers, too.

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